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A STUDY OF THE VENOM OF RENARD'S VIPER

Vipera ursini renardi

II. CHEMICAL AND BIOLOGICAL CHARACTERIZATION OF THE FRACTIONS

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Of the five fractions obtained as the result of the fractionation of the venom of Renard's viper on Sephadex G-75, the first two possessed a high proteolytic and coagulating activity and had a hemorrhagic and lethal action, and the third fraction has been identified as phospholipase A_2.

The separation of the venom of Renard's viper Vipera ursini renardi Ch. into its individual components and the comparative investigation of their chemical and biological properties is the most rational approach to the solution of problems connected with the elucidation of the mechanism of the action of the venom and its use in scientific and medical practice. Information has been published previously [1] on the chemical composition and biological action of the whole viper venom. The present paper gives the results of fractionation of the viper venom on Sephadex and of a study of the chemical properties and biological effects of the fractions obtained in comparison with those of the whole venom.

When the whole viper venom was passed through Sephadex G-75 gel, it was separated into five fractions (Fig. 1). Proteolytic activity was detected mainly in fractions I and II, which left the dextran gel column in a volume of eluent of 150 ml. In view of the fact that fractions I and II begin to emerge immediately after the passage of the free volume of the column (130 ml), in accordance with the theory of gel filtration [2] it may be assumed that the proteolytic activity of the venom is connected with components having molecular weights of 15,000-25,000 dalton. Phospholipase A_2 was separated from the proteolytic enzymes and appeared in fraction III, containing substances with molecular weights of 15,000 and below. These three fractions compose the bulk of the whole viper venom and are characterized by a predominantly protein composition:

	Fraction				
	I	*II*	*III*	*IV*	*V*
Yield, %	29,2	23,2	22,1	5,1	18,1
Protein according to Lowry 83	92,0	93,0	98,0	37,0	37,0

The yields of fractions IV and V were only 5.1 and 18.1%, respectively, and their content of protein substances was extremely low. It may be assumed these fractions contained low-molecular weight components (peptides of different sizes, free amino acids, catecholamines) similar to those obtained from the venom of the Bulgarian viper [3]. On the whole, the results of gel filtration show that the viper venom that we are investigating differs fundamentally in the ratio of high- and low-molecular-weight components from the venoms of the Elapidae [4, 5] and, according to the chemical classification of snake venoms [6], it can be assigned to the high-molecular-weight group.

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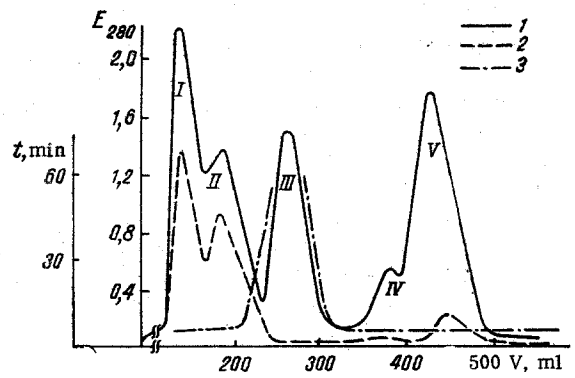

Fig. 1. Fractionation of 120 mg of venom on Sephadex G-75: 1) protein content (from the absorption at 280 nm); 2) proteolytic activity; 3) phospholipase A activity.

The results of investigations of the enzymatic activities and biological effects of the whole venom of Renard's viper and of the fractions obtained from it are given below:

	Toxicity	Protease, units	Fibrinolytic activity	Phospholipase, min	Indirect hemolysis, %	Coagulating activity, sec	Hemorrhagic activity, cm
Renard's viper venom	+	964	+	40	96,4	19	1,6
Fraction I	–	1540	–	7	0	58	1,3
II	+	1009	+	9	0	7	1,9
III	–	25	–	60	100	13	0,7
IV	–	70	–	8	0	16	–
V	–	422	+	7	0	20	–

Fractions I and II, containing proteolytic enzymes, were lethal for mice. The same fractions possessed the most powerful hemorrhagic action and fraction II, in addition, actively accelerated the clotting of citrate plasma from human donor blood. It is known that the hemorrhagic and coagulating effects of various snake venoms may in fact be connected with their proteolytic activity, and the results that we have obtained confirm this hypothesis [7]. The slight coagulating effect of fraction I is probably due to the predominance of proteases acting fibrinolytically in it. In general, the hydrolysis of fibrin and dissolution of a fibrin clot corresponding to this are functions of proteolytic enzymes. In actual fact, our results indicate that fibrinolytic activity is always accompanied by proteolytic activity, and it was even detected in fraction V the proteolytic action of which is possibly due to the presence of active fragments of proteases.

As was expected, fraction III, containing phospholipase A_2, caused the hemolysis of erythrocytes in the presence of an extra-erythrocytic source of phospholipids. It was established that this fraction also stimulated the clotting of blood and caused hemorrhage, although it cannot be excluded that it contains proteolytic enzymes not detected by the methods used in our work. A hemorrhagic action of phospholipase A_2 also appears possible and has been discussed in the literature [8, 9].

The results of disk electrophoresis indicated a high heterogeneity of the fractions obtained. Consequently, a definite identification of the active principles of Renard's viper venom that we are investigating does not yet appear possible. Nevertheless, it may be assumed that the coagulating and hemorrhagic activity of Renard's viper venom is connected in the first place with the activity of the proteolytic enzymes. This combination in individual fractions of hemorrhagic, coagulating, and proteolytic activity was also found in the separation of the venom of the viper V. ammodytes [10].

EXPERIMENTAL

The venom of the viper Vipera ursini renardii Ch. was obtained from the laboratory of the ecology of poisonous snakes of the Institute of Zoology and Parasitology, Academy of Sciences of the Uzbek SSR and from the Kirghiz Zoological Combine. We use Sephadex G-75 (Sweden) and a set of reagents for disk electrophoresis from Hungary, the other reagents being of ChDA ["pure for analysis"] grade.

Toxicity was determined by the intraperitoneal administration of the material to white mice weighing 18-20 g, the LD_{50} values and their upper and lower confidence limits being calculated by the method of Litchfield and Wilcoxon [11]. The hemorrhagic effect was evaluated by Kondo's method [12] 30 minutes after the administration of the material in a dose of 2.5 mg/kg. Proteolytic activity was determined by Kunitz's method on a casein substrate [13], phospholipase activity from the time of inhibition of the coagulation of egg yolk [14], and coagulating activity from the clotting of citrate human plasma [15].

For fractionation we used a column of Sephadex G-75 with dimensions of 25 × 850 mm. Elution was carried out with 0.05 M ammonium bicarbonate solution at the rate of 30 ml/h. The proteolytic and phospholipase activities of the samples were determined. The protein "peaks" were collected, concentrated, desalted, passed through Sephadex G-75 gel, and freeze-dried. Disk electrophoresis [16] was carried out in 7.5% polyacrylamide gel in buffer systems with pH 4.3 and 8.3.

SUMMARY

The venom of Renard's viper has been fractionated on Sephadex G-75. Of the five fractions obtained, the first two possessed high proteolytic and coagulating activities and had hemorrhagic and lethal effects, and the third fraction was identified as phospholipase A_2.

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